NM: Rose Allison [redacted]
TI: Maggie Johnson [redacted]

RAD Disposition Mtg: 06/10/09 [redacted]

RISK ASSESSMENT: PMN 09-0291

SUMMARY: The submitter intends to import this perfluoronated ammonium salt [redacted]. Based on data submitted with the PMN, and on structure [redacted] the PMN chemical should be relatively less persistent and more likely to be excreted than PFOA.
Human Health: Concerns are Systemic Tox (liver & adrenal) from 28-Day Oral Tox. repeat dose NOAEL of <0.5 mg/kg/day (Male rats) and 5 mg/kg/day (Female rats) rats dosed at 0, 0.5, 5 & 50 mg/kg/day, non-reversible liver & adrenal effects seen AND Repro/Developmental by analogy to PFOA.
Uncertainties: Without data it is difficult to estimate the extent of elimination. A pharmacokinetics study would help compare rate of excretion / elimination to that of PFOA.
Aquatic Toxicity: Low acute risk exists; CC not exceeded; releases 3 days/yr. Chronic testing recommended if future releases could increase.
Exposure/Risk: No consumer exposure is expected, and the only exposure of concern is occupational. The margins of exposure (MOE) are well within acceptable ranges, indicating no risk is expected.
Test Recommendations: Pharmacokinetics study to evaluate rate of excretion / elimination; Aquatic Tox Chronic studies IF surface water releases exceed current estimates.

I. BACKGROUND

Structure: (right) [redacted]

Formula: [redacted]

Identity: [redacted]

Categorical Name: [redacted] **CAS RN** [redacted]

Analogs: [redacted]

Submitter: [redacted] **Trade Name:** [redacted]

Prod. Vol. [redacted]

Process/Use: [redacted] polymerization [redacted]

P/Chem Properties: [redacted] WS=Disp (E): [redacted]

Concerns Identified at SAT (04/10/2009):
Health: 3 Dermal, Drinking Water, Inhalation, fish ingestion; Eco: 1
PBT rating: P3B3T3

Concerns Identified at FOCUS (04/23/09): Mutagenicity; acute toxicity, dermal irritation, corrosion to eyes, lungs, mucous membranes; blood, liver, thyroid, adrenal gland toxicity based on submitted test data; developmental neurotoxicity based on thyroid toxicity in dams; oncogenicity and immunotoxicity based on PFOA & PFOS; lung toxicity based on surfactancy

Toxicity Data:
Human Health Toxicity data (SAT Report 04/10/09):
- (-) Salmonella with and without activation;
- (-) E. coli with and without activation;
- (+) for chromosome aberrations in V79 cells with and without activation;
- rat oral LD50 >300 <2000 mg/kg;
- rat dermal LDO = 2000 mg/kg;
- slight skin irritation in rabbits;
- corrosive to the eye in rabbits;
- (-) for skin sensitization in a mouse local lymph node assay at conc. up to 25% ai;
- rat 14-d oral LOEL = 0.5 mg/kg/d, blood and liver toxicity;
- rat 28-d oral LOEL = 0.5 mg/kg/d, blood, liver, thyroid, and adrenal gland toxicity

Aquatic Toxicity data provided indicates low concern for acute toxicity & risk to Fish, Daphnid, Green Algae; all tests considered valid; CC of 1000 ppb not exceeded; if releases exceed the estimated maximum of [redacted] kg/site/day (releases for [redacted] chronic testing is recommended. [redacted]

II. HEALTH ASSESSMENT SUMMARY

Absorption/Metabolism (See attached memo from Dr. Keifer)
- Estimated absorption Skin <1%; Lung and GI 100%
- Pharmacokinetic studies (found in literature) on PMN substance in rats, mice, monkeys showed:
  - PMN has significantly longer half-life in male rats than in female rats;
  - Shows a trend toward accumulation in plasma in mice; and
  - Insufficient data to determine accumulation in monkey test, despite slow excretion of PMN in one monkey

Human Health Acute Toxicity, Systemic Toxicity, Irritation / Corrosion (See attached memo from Dr. Russell) The NOAEL of <0.5 mg/kg/day, rat 28-Day Oral Tox Repeat Dose Study will be used to calculate MOEs
Acute Toxicity, Oral
- LD50 of 300 mg/kg/bw in Acute Oral Toxicity in female rats (OECD 420)

Acute Toxicity, Dermal
- LD50 of >2,000 mg/kg/bw in Acute Dermal Toxicity in male & female rats (OECD 402), single dose, 24-hr, semi-occluded application
- Mild Dermal Irritation (redness) shown in Acute Dermal Toxicity in rabbits (OECD 404), single, 4-hr, semi-occluded application, skin normal at 72-hrs

Irritation / Corrosion
- Corrosive, Irreversible Eye Irritation shown in rabbit (OECD 405) single application

Systemic Toxicity Repeat Dose
- NOAEL <0.5 mg/kg/day (Male rats) and 5 mg/kg/day (Female rats) 14-Day Oral Toxicity in male & female rats dosed at 0, 0.5, 5 & 50 mg/kg/day
- **NOAEL <0.5 mg/kg/day (rats ♂)** and 5 mg/kg/day (rats ♀) 28-Day Oral Toxicity in male & female rats dosed at 0, 0.5, 5 & 50 mg/kg/day, non-reversible liver & adrenal effects

Immunotoxicity (See attached memo from Dr. Ward)
- Not a skin sensitization hazard based on a Local Lymph Node Assay of PMN;
- Some potential to be an immunotoxicity hazard by analogy to PFOA and PFOS (published animal studies);
- Potential to suppress immune response and augment the IgE response to environmental antigens, with considerable strain and species variability in the magnitude of the immune-related responses in the animal models. More research is needed to determine how immunotoxic PFOA and PFOS are in humans.

Mutagenicity (See attached memo from Dr. Cimino)
- Not a mutagenicity concern based both on submitted data and analog data

Oncogenicity (See attached memo from Dr. Woo)
Marginal concern for cancer by comparison to PFOA. NOTE: Based on [redacted] PMN chemical should be relatively less persistent and more likely to be excreted than PFOA. However, without data it is difficult to estimate the extent of elimination. **A pharmacokinetics study would help compare rate of excretion / elimination to that of PFOA.**

Reproductive / Developmental Toxicity (See attached memo from SRC)
For comparison, the following Reproductive /Developmental NOAELs were reported in the literature for PFOA (which the PMN will be replacing) and its salt APFO:
- Repro. & Dev. NOAEL 0.3 mg/kg/day for APFO - female mice dosed on Gestation Days 1-17
- Dev. & Maternal tox. LOAEL 1 mg/kg/day for PFOA - female mice also dosed on Gestation Days 1-17
- Repro. Tox. NOAEL 30 mg/kg/day; Dev. Tox. NOAEL 10 mg/kg/day for PFOA, 2-gen study in rats

Aquatic Toxicity (See attached memo from Dr. Pollack)
- Results from fish, Daphnid and green algae acute tests are considered valid
- Low aquatic toxicity and low risk - CC not exceeded [redacted]
- Chronic testing recommended if releases exceed predicted parameters

III. RISK ASSESSMENT - HEALTH

Toxicity Data on PMN Chemical: 28-Day Oral Toxicity, repeat dose **NOAEL of <0.5 mg/kg/day** (Male rats) and 5 mg/kg/day (Female rats) rats dosed at 0, 0.5, 5 & 50 mg/kg/day, non-reversible liver & adrenal effects seen.

Assumptions (identified at FOCUS)
Effects of Concern – Reproductive, developmental
Routes of Concern – Dermal, Inhalation
Populations of Concern – Occupational (no consumer)

Exposure Scenarios (See attached Exposure Results Summary Table, from J. Kwon)
[redacted]

[redacted]

Risk Calculations Assumptions:
Absorption 100% via lungs, Concerns are is repro & developmental; PMN <0.1% in material;
NOAEL is **<0.5 mg/kg/day**, Risk exists if MOE (NOAEL/exposure dose) <100
Calculation = ADR x Abs x %PMN = Actual ADR, NOAEL/Actual ADR = MOE

Occupational Inhalation – NO Risk for ADR of [redacted] or for ADR [redacted]

	value	units		value	units
ADR (Acute Dose Rate)	[redacted]	**mg/kg/day**	ADR (Acute Dose Rate)	[redacted]	**mg/kg/day**
absorption factor	100%		absorption factor	100%	
% PMN present	0.10%		% PMN present	0.10%	
Actual ADR	[redacted]	mg/kg/day	Actual ADR	[redacted]	mg/kg/day
NOAEL	0.5	mg/kg/day	NOAEL	0.5	mg/kg/day
MOE	[redacted]		MOE	[redacted]	

Estimated Consumer Risk – No Consumer exposure or risk

Health, Cancer Risk - Marginal cancer risk (see Sec II)

Health, Non-cancer Risk – Calculations indicate that no potential risk exists

V. TEST RECOMMENDATIONS

Human Health Toxicity: Oncogenicity (See attached memo from Dr. Woo)
Marginal concern for cancer by comparison to PFOA. NOTE: Based on [redacted] the PMN chemical should be relatively less persistent and more likely to be excreted than PFOA. However, it is difficult to estimate the extent of limitation. **A pharmacokinetics study would help compare rate of excretion / elimination to that of PFOA.**

Aquatic Toxicity: Chronic testing is recommended for PMN P09-0291 if there is the possibility that the volumes released into the environment may increase and thus introduce greater surface water concentrations in the future. EPA recommends the following testing and stipulations:

- OPPTS 850.1400 (Fish Early Life-Stage) Flow-through method, analytical measurement of test substance;
- OPPTS 850.1300 (Chronic Daphnia) Flow-through method, analytical measurement of test substance;
- OPPTS 850.5400 (Algae) Analytical measurement of test substance;
- Submission to EPA of the proposed test protocols for review and comment (and modification if necessary) prior to initiating testing; and
- Provide certificate of analysis for the tested material.

VI. SUBSTITUTES / BENEFITS [pending trigger information from submitter]

ATTACHMENTS

MEMORANDUM

SUBJECT: Absorption Standard Review and Study Review for PMN 09-0291

FROM: Leonard C. Keifer, Ph.D., FAIC
Chemist
New Chemicals Screening and Assessment Branch
Risk Assessment Division (7403M)

TO: Maggie Johnson, Ph.D.
Technical Integrator
New Chemicals Screening and Assessment Branch
Risk Assessment Division (7403M)

THRU: Robert E. Morcock, Ph.D., Chief
New Chemicals Screening and Assessment Branch
Risk Assessment Division (7403M)

[redacted]

I. INTRODUCTION

PMN substance 09-0291, [redacted] dispersible in water (SAT Report).

II. CONCLUSIONS

A. Absorption:

Estimated percent absorbed:
SKIN: <<1%
LUNG: 100%*
GI TRACT: 100%*

* Lung and GI Tract absorption percentages are valid only for very low doses (see below).

[redacted]

III. BASES FOR CONCLUSIONS

A. Absorption:

1. Skin:

[redacted] (an analogue for the PMN substance) was applied to skin that had been clipped free of hair as a suspension in water at 5,000 mg/kg covering 40% of the total body surface area often male and ten female rabbits. An impervious plastic sheet occluded the skin for 24 hours and was then removed. Animals were maintained and observed for a 28-day period. Blood samples were obtained on days 1, 7, 14, and 28. Analysis for total blood fluoride was performed on the day one and day 28 samples from a single male and single female.

Total serum fluoride values for the male were 10.3 ppm for day one and 130 ppm for day 28. The respective values for the female were 0.9 ppm and 128 ppm. Although this study indicated some dermal absorption, it is limited in that the values from only two animals were measured, and only from the day one and day 28 samples [redacted]

Three male and three female albino rabbits per dose group were exposed dermally under occlusion for 24-hours to an aqueous suspension of [redacted] (0.06 %) at doses equal to 0, 0.003, 0.06, and 0.3 mg [redacted]/kg and held for 28 days ([redacted] Liver samples taken at term were analyzed for total organic fluorine. No quantifiable organic fluorine could be detected in the 28-day livers by combustion or by electrospray mass spectroscopy [redacted]

2. Lung:

No information was found on close analogues of the PMN substances. Lung absorption is judged to be equal to GI absorption.

3. GI Tract:

At least 95% of a single oral dose averaging 4.2mg/kg [redacted] administered to two groups (24 hour and 48 hour sacrifice) of 3 male [redacted] CD rats (248-315 g, mean = 285 g) was absorbed within 24 hours [redacted]. The radiochemical purity of the [redacted] used in this study listed below was >99% [redacted]

IV. PK STUDY REVIEWS

A. Single Oral Dose in Rats: Groups of 5 male and 5 female Crl:CD(SD) rats were given single oral gavage doses of 20 mg/kg of the PMN substance. Blood samples were drawn prior to dosing and at 15 minutes, and 1, 2, 4, 8, 24, 48, 96, 168, 336, and 504 hours post-dosing. Results are shown in the Table 1 [redacted]

Table 1. Pharmacokinetic Parameters for PMN substance in Rats							
Sex	Cmax ug/mL	Tmax h	t1/2 h	AUClast ugh/mL	AUCinf ugh/mL	CL/F mL/h/kg	Vd/F mL/kg
Male (SD)	123 9.5	4	59 16	6973 1077	7045 1079	2.9 0.4	240 40
Female (SD)	122 40	2	1.2 0.1	376 128	381 130	57 17	92 24

B. Single and Multiple Oral Dose in Mice: Groups of 4 male and 4 female Crlj:CD1(ICR) mice were dosed via oral gavage with the PMN substance as shown in the following list:

5mg/kg X 1
5mg/kg X 7
5mg/kg X 14

20 mg/kg X 1
20 mg/kg X 7
20 mg/kg X 14

A blood sample was drawn from each animal 24 hours after the last oral dose. Plasma concentration of the PMN substance was [redacted] each sample. Results are presented in Table 2 [redacted]

Table 2. Plasma concentration of PMN Substance in mice following 1, 7, or 14 Doses	
Dose group (mg/kg)	Plasma Conc [ug/mL (SD)]
5 X 1	14.0 (1.2)
5 X 7	86.8 (16.9)
5 X 14	115.7 (25.0)
20 X 1	53.8 (6.1)
20 X 7	52.1 (3.5)
20 X 14	133.5 (16.5)
5 X 1	13.8 (8)
5 X 7	36.4 (5.2)
5 X 14	32.5 (15.4)
20 X 1	36.0 (10.1)
20 X 7	52.3 (11.6)
20 X 14	100.3 (18.5)

C. Single Oral Dose in Monkeys: Four male Cynomolgus monkeys were each given a single iv dose of 10 mg/kg of PMN substance. Blood samples were drawn prior to dosing and at 1, 4, and 8 hours and 1, 2, 4, 7, and 14 days after dosing. Results are shown in the Table 3; half-life and area under the curve were calculated [redacted] 4 days and using the data for the first 48 hours

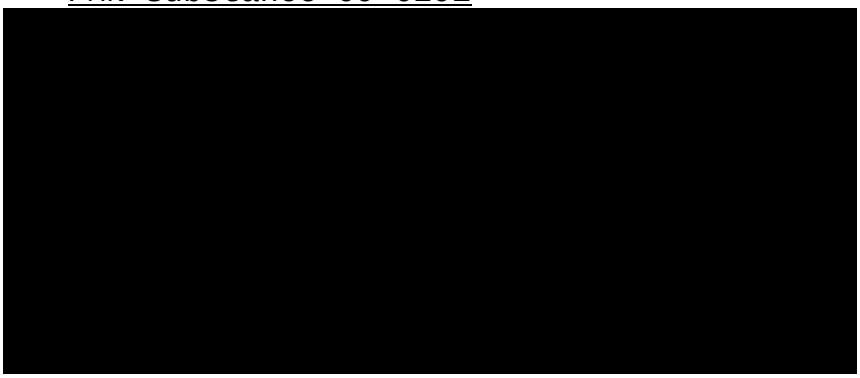
Table 3. Pharmacokinetic Parameters for PMN substance in Monkeys		
	At 14 days	At 48 hours
t1/2 (h)	25.5 (12.8)	18.4 (5.5)
AUCinf (ugh/mL)	2021 (893)	1524 (411)
CLtotal (mL/h/kg)	5.6 (2.1)	

[redacted] ewer's Discussion: In the single dose study in rats [redacted] the half-life in plasma was determined to be significantly longer on male rats than in females.

In the study in mice [redacted] sufficient samples were not taken to determine the half-life in plasma, however, examination of the plasma levels of PMN substance is judged to show a trend toward accumulation in the animals.

The monkey study [redacted] does not provide sufficient data to determine if the PMN substance will accumulate in the animals. The 25.5 hour half-life at 14 days was influenced by the very slow excretion of the iv dose by one animal.

PMN Substance 09-0291



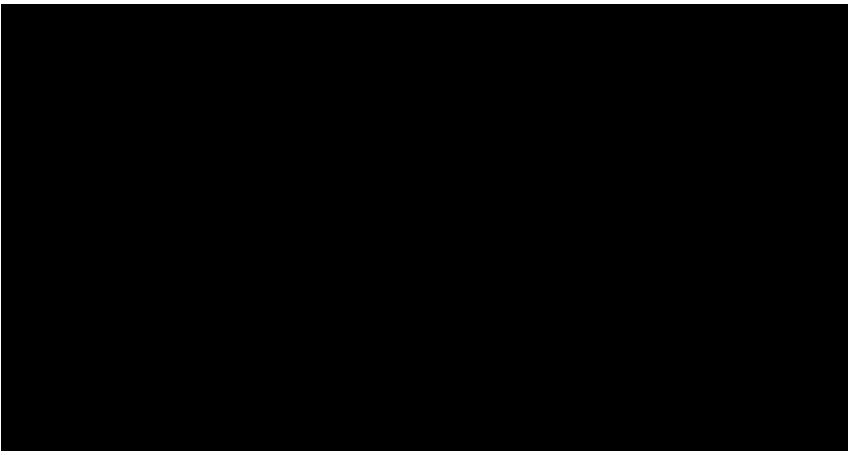


Figure 1. Structures of PMN Substance 09-0291 and an Analogue.

REFERENCES

[redacted]

MEMORANDUM **May 12, 2009**

[redacted]

SUBJECT: Review of data on Acute Oral Toxicity, Acute Dermal, Systemic Toxicity (Repeated Dose 14-Day Toxicity and Repeated Dose 28-Day Toxicity), and Irritation/ Corrosion of chemical substance [redacted] (P09-291).

FROM: Lemuel T. Russell IV
Toxicologist
New Chemicals Screening and Assessment Branch
Risk Assessment Division (7632)

TO: Maggie Johnson
Biologist
New Chemicals Screening and Assessment Branch
Risk Assessment Division (8924)

THRU: Bob Morcock
Chief
New Chemicals Screening and Assessment Branch
Risk assessment Division (8907)

I. CONCLUSIONS:

The data reviewed below adequately meet the TSCA requirements for Acute Oral Toxicity, Acute Dermal, Systemic Toxicity (Repeated Dose 14-Day Toxicity and Repeated Dose 28-Day Toxicity), and Irritation/ Corrosion of chemical substance [redacted] (P09-291).

Conclusion Summary:

The acute LD_{50}, 300 mg/kg/bw of the test substance (P09-291) in female Sprague Dawley CD rats is consistent with a low degree of toxicity. The acute dermal LD_{50} of P09-291 in the Sprague-Dawley CD rats is also consistent with a low degree of toxicity. Additionally, P09-291 produced a primary irritation index of 0.3 and was classified as a mild irritant according to the Draize classification scheme. The test substance P09-291 also produced irreversible ocular damage and was corrosive to the rabbit eye. A 14-Day and a 28-Day repeated dose toxicity study concluded that the NOEL of test substance P09-291was less than 0.5 mg/kg/day in the male rats and 5 mg/kg/bw in female rats.

II. BASIS OF CONCLUSIONS:

The PMN test substance is a [redacted] (PMN submission).

a. Acute Oral Toxicity

This acute oral toxicity testing of test substance (P09-291) is consistent with OECD guidelines No. 420. Following a sighting test at dose levels of 300 mg/kg and 2000 mg/kg, an additional group of four female Sprague-Dawley CD rats were given a single dose of the test material, as a solution in distilled water, at a dose of 300 mg/kg bodyweight.

Clinical signs and bodyweight modulations were monitored during the study and all animals were subject to gross necropsy.

Mortality. No deaths were noted at dose level 300 mg/kg/bw and the animal treated with a dose level of 2000 mg/kg bodyweight was in extremis one day after dosing. No deaths were observed at the dose level of 300 mg/kg.

Clinical Observations**.** Clinical observations observed in the animal treated with a dose level of 2000 mg/kg bodyweight included hunched posture, lethargy, ataxia, pilo-erection, and occasional body tremors and hypothermia. There were no clinical signs observed in animals treated at a dose level of 300 mg/kg/bw.

Bodyweight. All surviving animals showed normal bodyweight gains.

Pathology. A patchy pallor of the liver was observed at necropsy of the animal treated with a dose level of 2000 mg/kg that was killed in extremis. Additionally no abnormalities were observed in animals that were killed at the end of the study.

Conclusion. The acute oral median lethal dose (LD_{50}) of 300 mg/kkg/bw of the test substance in the female Sprague-Dawley CD (Globally Harmonised Classification System—Category 4) is consistent with a low degree of toxicity.

b. Acute Dermal Toxicity (rat)

This acute dermal toxicity testing of test substance-neat (P09-291) is consistent with OECD guidelines No. 402. A group of ten Sprague-Dawley CD rats (five male and five females) were given a single, 24 hour, semi-occluded dermal application of the test substance-neat (P09-291) to intact skin at a dose of 2000 mg/kg bodyweight.

Clinical signs and bodyweight modulations were monitored during the study and all animals were subject to gross necropsy.

Mortality. No deaths occurred.

Clinical Observations. No clinical observations were observed.

Dermal Irritation. No dermal irritation was observed.

Bodyweight. Animals showed normally expected gains in bodyweight gains.

Pathology. There were no abnormalities noted at necropsy.

Conclusion: The acute dermal median lethal dose (LD_{50}) of > 2000 mg/kg/bw of the test substance (P09-291) in the Sprague-Dawley CD rats is consistent with a low degree of toxicity.

c. Acute Dermal Toxicity (rabbit)

The acute dermal toxicity testing of test substance (P09-291) is consistent with OECD guidelines No. 404 " Acute Dermal Irritation/ Corrosion". A single 4-hour, semi-occluded application of the test substance (P09-291) dissolved in water to intact skin of three New Zealand White rabbits produced a very slight erythemaa (degree of redness not specified) at two treated skin sites. Two skin sites appeared normal at the 72-hour observation and the remaining skin site appeared normal throughout the study.

Conclusion: The test substance (P09-291) produced a primary irritation index of 0.3 and was classified as a mild irritant according to the Draize classification scheme.

d. Acute Eye Irritation

The acute dermal toxicity testing of test substance (P09-291) is consistent with OECD guidelines No. 405 "Acute Eye Irritation/ Corrosion". A single application of the test substance-neat (P09-291) to the non-irrigated eye of one New Zealand White rabbit produced scattered or diffused corneal opacity, iridial inflammation and moderate conjunctival irritation. Additional effects observed were vascularization, with localized ingrowth of vessels, hemorrhage of the upper nicitation membrane and palpebral conjunctival membrane and alopecia around the eye. A persistence of the reactions in the treated eye at the 21-day observation was believed to be indicative of irreversible ocular damage.

Conclusion: The test substance (P09-291) produced irreversible ocular damage and was considered to be corrosive to the rabbit eye.

e. Systemic Toxicity (Repeated Dose 14-Day Toxicity)

A 14-Day repeated dose toxicity study was conducted to evaluate systemic toxicity of test substance (P09-291) dissolved in purified water, purity (99.3%). The test substance (P09-291) was administered repeatedly by oral gavage at doses of 0, 0.5, 5 and 50 mg/kg/day to male and female Crl:CD(SD) rats for 14 days. The study concluded that the no observed effect level (NOEL) of test substance (P09-291) was less than 0.5 mg/kg/day in the male rats and 5mg/kg/day in female rats.

f. Systemic Toxicity (Repeated Dose 28-Day Toxicity**)**

A 28-Day repeated dose toxicity study was conducted to evaluate systemic toxicity of the test substance (P09-291) dissolved in purified water, purity (99.3%). The test substance (P09-291) was administer repeatedly by oral gavage at doses of 0, 0.5, 5 and 50 mg/kg/day to male and female Crl:CD(SD) rats for 28 days. The study concluded that the no observed effect level (NOEL) of test substance (P09-291) was less than 0.5 mg/kg/day in the male rats and 5mg/kg/day in female rats.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

05/28/09

MEMORANDUM

SUBJECT: P09-291 Immunotoxicity Standard Review

FROM: Ronald E. Ward Ph.D., Microbiologist/Immunologist
RAD/SSB (7403M)

THRU: Donald Rodier, Branch Chief
RAD/SSB (7403M)

TO: Maggie Johnson, Technical Integrator

[redacted]

Conclusion: Based on a submitted LLNA study, PMN 09-291 [redacted] is not a skin sensitization hazard. Based on (PFOA and PFOS) analog data from published animal studies, this PMN would have some potential to be an immunotoxicity hazard. This PMN would have the potential to suppress the immune response and augment the IgE response to environmental antigens. However, there seems to be considerable strain and species variability in the magnitude of the immune-related responses in the animal models. More research is needed to determine how immunotoxic PFOA and PFOS are in humans.

Basis for Conclusion:

There was one (LLNA) (1) report submitted with the PMN. The PMN tested negative in the LLNA assay. Therefore, the PMN substance is not a skin sensitization hazard.

There were no other direct immunotoxicity test data associated with this PMN. The following conclusions reached here are based on analog test data.

There is a concern for immunotoxicity based on PFOA (perfluorooctanoic acid) as analog. Based on four earlier published studies by Yang et al., PFOA is immunotoxic in mice (2,3,4,5). Feeding C57Bl/6 mice a diet containing 0.02% PFOA resulted in adverse effects to both the thymus and spleen. In addition, this feeding regimen resulted in suppression of the specific humoral immune response to horse red blood cells, and suppression of splenic lymphocyte proliferation in response to LPS and ConA. The suppressed mice recovered their ability to generate a humoral immune response when they were fed a diet devoid of PFOA. Studies using transgenic mice showed that the peroxisome proliferator-activated receptor alpha was involved in causing the adverse effects to the immune system.

In a May 2008 publication, DeWitt et al. (6) examined PFOA effects on humoral and cellular immunity using standard assays for assessing immune function, and also derived dose–response data. To perform the dose response experiment, groups of C57BL/6N female mice (8 animals per dose group) were given PFOA in drinking water for 15 days. The resulting PFOA doses were 30, 15, 7.5, 3.75, 1.88 and 0.94 mg/kg/day, based on average daily water consumption rates. Mice were immunized with sheep red blood cells in Freund's complete adjuvant on day 11 of exposure; immune responses were determined one day post-exposure.

The results showed that SRBC-specific IgM synthesis (ELISA titers) was suppressed at exposures ≥ 3.75 mg PFOA/kg/day in a dose-dependent manner. A NOAEL for suppressed IgM production was identified as1.88 mg PFOA/kg/day and a LOAEL for suppressed IgM production was 3.75 mg PFOA/kg/day. The conclusion of these experiments was that IgM antibodies were suppressed after PFOA exposure.

In a June 2008 article (7), Loveless et al. tested ammonium perflourooctanote (APFO) for immunotoxicity in both mice and rat models. To perform the experiments, male CD rats and CD-1 mice were dosed by oral gavage with 0.3 to 30 mg/kg/day of ammonium perfluorooctanoate **(**APFO) for 29 days. The authors then evaluated the anti-sheep red blood cell IgM levels, clinical signs, body weights, selected hematology and lipid parameters, liver weights, spleen and thymus weights and cell number, selected histopathology, and serum corticosterone concentrations.

The results in mice showed that there was decreased IgM antibody production at 10 (20% suppression) and 30 mg/kg/day (28% suppression); decreased spleen and thymus weights and cell numbers; microscopic depletion/atrophy of lymphoid tissue at 10 mg/kg/day (thymus) and 30 mg/kg/day (spleen). The authors suggest that these immune-related findings were likely secondary responses to systemic toxicity (loss of body weight) and stress observed at these doses. The authors also report the NOAEL for APFO systemic toxicity in male mice to be 0.3 mg/kg/day based on liver necrosis at 1 mg/kg/day. They also report the NOAEL for immunotoxicity to be 1 mg/kg/day, based on suppression of anti-SRBC response.

However, in CD rats, Loveless et al.(7) reported that APFO had no effect on the production of anti-SRBC antibodies. Thus, they concluded there was no immune-related changes occurred in rats, even at doses causing systemic toxicity.

In a March 2008 article (8), Peden-Adams et al. tested perfluorooctane sulfonate (PFOS) for immunotoxicity in B6C3F1 mice. These authors exposed adult male and female B6C3F1 mice to perfluorooctane sulfonate (PFOS) daily via gavage for 28 days(0, 0.005, 0.05, 0.1, 0.5, 1, or 5 mg/kg total-administered dose[TAD]). Following exposure, various immune parameters were assessed and serum PFOS concentrations were determined.

The results showed that the anti-sheep red blood cell plaque forming cell (PFC) response was suppressed in male mice beginning at 0.05 mg/kg TAD and in females at 0.5 mg/kg TAD. In addition, the serum trinitrophenyl (TNP)–specific IgM titers were also decreased by PFOS after TNP–LPS (TNP conjugated to lipopolysacharide) challenge suggesting that the humoral immune effects may be attributed to the B-cell rather than T-cell because both T-dependent (SRBC) and T-independent (TI) (TNP–LPS) antigens result in suppressed IgM production. Based on the PFC response, the low observed effect level (LOEL) for males was reported to be 0.05 mg/kg TAD and for females was 0.5 mg/kg TAD.

In addition to immunosuppression, there is also evidence in the literature that PFOA may influence IgE-dependent allergic asthma. Fairley et al.(9) investigated the role of dermal exposure to perfluorooctanoic acid (PFOA) on the hypersensitivity response to ovalbumin (OVA) in a murine model of asthma. These investigators exposed BALB/c mice

dermally, on the dorsal surface of each ear, to concentrations of PFOA ranging from 0.01 to 1.5% (applied dose 0.25–50 mg/kg) for four days. For the hypersensitivity studies, mice were also ip injected with 7.5 mg OVA and 2 mg alum on days one and 10 and in some studies challenged with 250 mg OVA by pharyngeal aspiration on days 17and 26.

The results showed that, compared to the OVA alone–exposed animals, an increase in total IgE was demonstrated when mice were coexposed to OVA and concentrations of PFOA ranging from 0.75 to 1.5%, with the OVA-specific IgE response peakng with 0.75% PFOA coexposure. OVA-specific airway hyperreactivity was increased in the 1.0% PFOA coexposed group, with an increased pleiotropic cell response characterized by eosinophilia and mucin production. In this murine model, PFOA was demonstrated to be immunotoxic following dermal exposure, with an enhancement of the hypersensitivity response to OVA, suggesting that PFOA exposure may augment the IgE response to environmental allergens.

A developmental immunotoxicity study of PFOS was performed by Keil et al.(10). These authors evaluated immunotoxicity in B6C3F1 pups following oral maternal exposure to PFOS on gestations days 1–17. Exposure levels included 0.1, 1, and 5 mg/kg/day PFOS. Natural killer (NK) cell activity, anti-SRBC IgM plaque assay, CD4/8 lymphocytic subpopulations, nitrite production in peritoneal macrophages, and body/organ weights were evaluated at four and 8 weeks of age in F1 pups.

The results showed that there were no significant dose-responsive changes in maternal or pup body weights, or macrophage function observed, yet hepatomegaly was indicated in F1 male pups at 4 weeks of age. Functional deficits were not evident until 8 weeks of age when NK cell function and IgM production were significantly decreased. When compared with females, male pups were more sensitive to the effects of PFOS. Based on functional immunological assays, the authors report a NOAEL and LOAEL of 0.1 and 1.0 mg/kg/day (males only) following maternal PFOS exposure level, respectively. This study establishes that the developing immune system is sensitive to the effects of PFOS and results in functional deficits in innate and humoral immunity detectable at adulthood.

In summary, based on a submitted LLNA study, PMN 09-29 [redacted] is not a skin sensitization hazard. Based on (PFOA and PFOS) analog data from published animal studies, this PMN would have some potential to be an immunotoxicity hazard. This PMN would have the potential to suppress the immune response and augment the IgE response to environmental antigens. However, there seems to be considerable strain and species variability in the magnitude of the immune-related responses in the animal models. More research is needed to determine how immunotoxic PFOA and PFOS are in humans. The C8 panel (11) is addressing the relationship between PFOA human exposure and indicators of immunotoxicity and incidence of infectious disease and asthma.

References

1. Kimber, I, and Weisenberger, C. A A murine local lymph node assay for the identification of contact allergens. Assay Development and Results of Initial Validation Study. Archives of Toxicology 1989 63: 274-282

2.Yang, Q., Xie, Y., and Depierre, W. 2000. Effects of peroxisome proliferators in the thymus of spleen and mice. Clin. Exp. Immunol. 122:219-226.

3. Yang, Q., Xie, Y., Ericksson, A.M., Nelson, B.D., and DePierre, J. W. 2001. Further evidence for the involvement of inhibition of cell proliferation and development in thymic and splenic atrophy induced by the peroxisome proliferator perfluoroctanoic acid in mice. Biochem. Pharmacol. 62: 1133-1140.

4. Yang, Q., Xie, Y., Alexson, S.E.H., Nelson, B.D., and DePierre, J. W. 2002a. Involvement of

the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. Biochem. Pharmacol. 63: 1893-1900.
5. Yang, Q., Abedi-Valugerdi, M., Xie, Y. Zhao, X., Moller, G., Nelson, B.D., and DePierre, J. W. 2002b. Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. International Immunopharmacology 2, 389-397.
6.DeWitt, J. C, et al. 2008. Perfluorooctanoic Acid-Induced Immunomodulation in Adult C57BL/6J or C57BL/6N Female Mice. Environmental Health Perspectives 116:644-650.
7. Loveless, S.E. et al. 2008, Evaluation of the Immune System in Rats and Mice Administered Linear Ammonium Perfluorooctanoate. Tox. Sci. 105 (1), 86-96.
8. Peden-Adams, M.M. et al. (2008) Suppression of Humoral Immunity in Mice following Exposure to Perfluorooctane Sulfonate. Tox. Sci. 104: 144-154.
9. Fairley, K.J. et al. (2007) Exposure to Immunosuppressant, Perfluorooctanoic acid, enhances the Murnine IgE and Airway Hyperreactivity Response to Ovalbumin. Tox. Sci. 97:375-383.
10. Keil, D.E., et al. (2008) Gestational Exposure to Perfluorooctane Sulfonate Suppresses Immune Function in B6C3F1 Mice. Tox. Sci. 103:77-85.
11. Fletcher, T., et al. (C8 panel update 2009) PFOA and Immune Biomarkers in Adults Exposed to PFOA in Drinking Water in Mid Ohio Valley.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

May 11, 2009

MEMORANDUM

SUBJECT: Mutagenicity Hazard Review of P09-291

FROM: Michael C. Cimino, Ph.D.
Biologist
Science Support Branch
Risk Assessment Division (7403M)

TO: Margaret Johnson, Ph.D.
Technical Integrator
New Chemical Screening and Assessment Branch (7403M)

THRU: Donald Rodier
Branch Chief
Science Support Branch
Risk Assessment Division (7403M)

[redacted]

I. CONCLUSION

The PMN is not a gene mutagen in two species of prokaryotes either without or with activation, and is a weak chromosomal mutagen in mammalian cells *in vitro* with activation but not without. There is weak concern for mutagenicity of the PMN. This weak mutagenicity concern does not reduce concern for carcinogenicity based upon other (non-genotoxicity) data, should such concern exist.

II. STRUCTURES OF P09-291 AND ANALOGUE

P09-291

III. BASIS FOR THE CONCLUSIONS

Mutagenicity data were provided with the Premanufacturing Notice on P09-291 These data are reviewed below.

I. Bacterial reverse mutation

P09-291 was tested in a bacterial reverse mutation assay, as reported in "Bacterial reverse mutation test of)", conducted by dated November 4, 2008. The test material (purity 99.6%) was identified as " as above for the PMN). It was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and in *Escherichia coli* strain WP2*uvrA*, both

without and with metabolic activation using phenobarbital- and 5,6-benzoflavone-induced Sprague-Dawley rat liver S9. It was tested in a preliminary test at seven dose levels ranging from 1.22 to 5,000 µg/plate. Two independent repeats were conducted at six dose levels of 156, 313, 625, 1,250, 2,500 and 5,000 µg/plate. Both repeats were conducted in triplicate plates. Bacterial growth was inhibited at dose levels of 2,500 µg/plate and above. Dose selection was acceptable. The chemical did not induce significant increases in gene mutations under any test condition. Concurrent negative (the solvent, dimethyl sulfoxide) and positive controls produced appropriate responses.

II. *In vitro* chromosome aberration

The PMN also was tested in an *in vitro* mammalian chromosome aberration assay, as reported in "Chromosome aberration study of [redacted]" in cultured mammalian cells", conducted by [redacted] dated October 29, 2008. The test material was identified as for the bacterial test, with purity as above. It was tested in Chinese hamster lung CHL/IU cells both without and with metabolic activation using rat liver S9, as above. A range-finding study at eight dose levels was conducted at dose levels from 39.1 to 5,000 µg/ml. Cytotoxicity was noted at dose levels of 1,400 µg/ml and above; 1,400 µg/ml was the highest dose employed in the subsequent mutagenicity assay proper. Duplicate flasks were used for each treatment group. Cells were exposed for six hours with 18h recovery, without and with activation, to dose levels of 1,000, 1,2000 and 1,400 µg/ml. Dose selection was acceptable. Increases in structural aberrations were noted for the highest dose without activation (10.5%), and for the medium (6.0%) and highest (17.0%) doses with activation. The highest dose in both activation conditions displayed approximately 65-67% cytotoxicity. The testing laboratory considered these three responses for structural aberrations to be positive. There were no significant increases in numerical aberrations (polyploidy). Concurrent negative (saline) and positive controls (mitomycin C and benzo[a]pyrene for non-activated and activated assays, respectively) produced appropriate responses. Since only one noncytotoxic dose produced mutagenicity (medium with activation, 6% aberrations), RAD concludes that the PMN is a weak chromosome mutagen in mammalian cells *in vitro* under the conditions tested.

In summary, P09-291 is not a gene mutagen in two species of prokaryotes either without or with activation, and is a weak chromosomal mutagen in mammalian cells *in vitro* with but not without activation.

Mutagenicity data also are available on analogues of the PMN:

(1) Data on a test material that is a [redacted] indicates that it is not a gene mutagen without or with activation in *Salmonella typhimurium* stains TA98, TA100, TA1535 and TA1537, or in *Escherichia coli* strain WP2*uvrA* (Cimino 2009b).

(2) Similarly, mutagenicity data on [redacted] indicate that it is not a gene mutagen without or with activation in *Salmonella typhimurium* stains TA98, TA100, TA1535 and

TA1537, or in *Escherichia coli* strain WP2*uvrA,* as reviewed at the SAT meeting for that case ([redacted]).

The PMN is not a gene mutagen in two species of prokaryotes either without or with activation. It is a weak chromosomal mutagen in mammalian cells *in vitro* with but not without activation. There is slight concern for the mutagenicity of the PMN. There is no other basis for a mutagenicity concern for large phosphates or perfluorooctyl (PFO) analogues. This weak mutagenicity concern does not reduce concern for carcinogenicity based upon other (non-genotoxicity) data, should such concern exist.

III. REFERENCES

[redacted]

From: Yin-tak Woo
To: Maggie Johnson
Thru: Don Rodier
Re: Cancer Concern for PMN P09-291

[redacted]

The PMN chemical P09-291 is the [redacted] A cancer concern was expressed at the SAT meeting by analogies to perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). Both PFOS and PFOA have been shown to be carcinogenic in rodents (see below) and persistent in exposed workers with serum half life of 4.8-5.4 years for PFOS and 3.5-3.8 years for PFOA (Olsen et al. Environ. Health Persp. 115, 1298, 2007).

Between the two analogs, PFOA is the better analogy for the PMN chemical. However, the analogy may be somewhat limited because [redacted] and the [redacted] These two changes should make the PMN chemical relatively less persistent and more likely to be excreted than PFOA. It is difficult to estimate the extent of limitation. A pharmacokinetics study will be most helpful in this respect.

Although PFOA has been shown to be carcinogenic, the concern for the cancer endpoint is substantially lower than that of developmental toxicity. PFOA has been/was given a *suggestive evidence* classification by EPA. The Science Advisory Board was indecisive with respect to whether PFOA should be suggestive or likely. PFOA has three cancer targets in rodents (liver, Leydig cells of the testis, acinar cells of the pancreas). The induction of rodent liver tumors is believed to be the result of activation of the peroxisome proliferator activated alpha receptor (PPARα) and is not considered to be of human significance. The Leydig cell tumors are hypothesized to be associated with an increased level of serum estradiol in concert with testicular growth factors. Both of these modes of action are of uncertain human relevance. The mode of action of the induction of pancreatic acinar cell tumors remains to be studied. No cancer risk assessment has been conducted by EPA. An earlier OPPT attempt of risk assessment of PFOA using mammary tumors was abandoned after the pathology re-reading of the slides changed the tumor incidence of the control group and obliterated the statistical significance.

Overall, considering the limited analogy to PFOA, and the mostly suggestive evidence of carcinogenicity of PFOA, the potential cancer concern for P09-291 is marginal and therefore should not be a driving point for quantitative risk assessment.

[redacted]

STANDARD REVIEW OF REPRODUCTIVE AND DEVELOPMENTAL TOXICITY FOR P-09-0291

I. INTRODUCTION

This report summarizes the potential adverse reproductive and developmental effects of PMN P-09-029 [redacted] based on reproductive and developmental toxicity data for perfluorooctanoic acid (PFOA) and its simple salts (analogs of the PMN substance).

Some of the data presented in this report were identified from two comprehensive review articles on perfluoroalkyl acids through 2007 (Lau *et al.*, 2004 and 2007). A search of TOXNET was performed to identify developmental and/or reproductive toxicity data on PFOA published post-2007. Key reproductive/developmental toxicity data for PFOA or its salt ammonium perfluorooctanoate, APFO (i.e., seemingly adequate studies that produced the most conservative LOAEL and NOAEL values), were reviewed and are described under section III (Basis for Conclusions) below. Other supplemental available reproductive/developmental toxicity data are briefly summarized below under section IV (Supplemental Data). The data presented in sections III and IV were previously reviewed in SRC's developmental/reproductive toxicity standard review for [redacted]

II. CONCLUSIONS

Since no reproductive or developmental studies were available for the PMN substance itself, the primary assessment of the potential of the PMN substance to produce reproductive and developmental toxicity is based on data for PFOA.

In an oral gavage study in mice by Abbott *et al.* (2007) with APFO, the developmental/reproductive toxicity NOAEL and LOAEL values appear to be 0.3 and 0.6 mg/kg/day, respectively, based on increased litter loss and decreased pup survival.[1] The maternal toxicity NOAEL and LOAEL values are 0.6 and 1 mg/kg/day, respectively, based on increased liver weights in dams.

In an oral gavage developmental toxicity study of PFOA in mice (Lau *et al.*, 2006), the LOAEL value for both maternal and developmental toxicity was 1 mg/kg/day (lowest dose tested) based on significantly increased absolute liver weights in dams (the study report did not provide liver-to-bodyweight ratios or liver histology data) and increased incidences of enlarged fontanel and reduced ossification of calvaria in pups at all dose levels. The number of dams with full litter resorption was increased at ≥5 mg/kg/day; this effect may be considered either a developmental or a reproductive effect.

[1] Increased relative liver weight occurred at ≥0.1 mg/kg/day in pups at weaning. Livers were not examined microscopically to determine if weight changes correlated with histological changes. In addition, the relevance of this finding to humans is not known (J. Seed, EPA, to Bill Richards, SRC, 4/13/2009).

If it is considered a reproductive effect, then the LOAEL and NOAEL values for reproductive toxicity would be 5 and 3 mg/kg/day, respectively.

In a 2-generation oral gavage reproductive toxicity study of APFO in rats (Butenhoff *et al.*, 2004), the LOAEL for systemic toxicity was 1 mg/kg/day (lowest dose tested) based on decreased mean body weight and increased absolute and relative liver weight in F_1 males, with evidence of liver histopathology at ≥3 mg/kg/day in the F_1 males. The NOAEL for reproductive toxicity was the highest dose tested (30 mg/kg/day) and the LOAEL and NOAEL values for developmental toxicity were 30 and 10 mg/kg/day, respectively, based on decreased mean pup weight and delayed sexual maturation.

III. BASIS FOR CONCLUSIONS

APFO (Abbott *et al.*, 2007)

The study used 129S1/SvlmJ wild-type mice (WT) and peroxisome proliferator-activated receptor alpha (PPAR-alpha) knockout mice (KO) to investigate whether PPAR-alpha mediates PFOA-induced developmental toxicity. Groups of WT and KO mice (group sizes not indicated) were administered APFO (>98% pure) by gavage in deionized water at dose levels of 0, 0.1, 0.3, 0.6, 1, 3, 5, 10, or 20 mg/kg/day on gestation days (GDs) 1-17. It appears that WT dams were exposed to 0, 0.1, 0.3, 0.6, 1, 5, 10, and 20 mg/kg/day, and KO dams were exposed to 0, 0.1, 0.3, 1, 3, 5, 10, and 20 mg/kg/day, but this is not clearly explained in the study report. At parturition, the number of live and dead fetuses was recorded and male and female pups were weighed (as a group/sex). The number of live pups in each litter was recorded and pups were weighed by sex on post-natal days (PNDs) 1-10, 14, 17, and 22. Eye opening was monitored beginning on PND12. On PND 22, pups were weighed and weaned and one pup per litter, plus all dams, were sacrificed (it appears that the one pup/litter was sacrificed in order to assess pup liver weight, although this is not clearly stated). Weaned pups were kept for further study and weighed monthly; males were monitored for up to 28 weeks of age, and females were monitored for up to 52 weeks of age (no further details provided). Additionally, to eliminate the possibility that maternal strain was a factor in pup survival, heterozygous litters (HET) were produced in PFOA-exposed WT and KO dams, and survival was monitored through PND15.

Maternal body weight and weight gain were not affected. For both WT and KO dams, the number of pregnant females at ≥5 mg/kg/day was much less than in controls (*e.g.*, 22, 12, 8, 16, 18, 6, 5, and 7 pregnant WT females at 0, 0.1, 0.3, 0.6, 1, 5, 10, and 20 mg/kg/day, respectively); however, because the study report did not indicate original group sizes at each dose level, this observation is difficult to interpret. Number of implantations was not affected. Percent litter loss ([no. implants – no. live pups]/no. implants x 100) was significantly increased in WT pups at ≥0.6 mg/kg/day and in KO pups at ≥5 mg/kg/day; full litter resorptions were seen at ≥5 mg/kg/day in both WT and KO dams. Pup birth weight was not affected in either mouse type. At weaning, relative liver weight was significantly increased at ≥0.1 mg/kg/day in WT pups, at 3 mg/kg/day in KO pups, at ≥1 mg/kg/day in WT dams, and at ≥3 mg/kg/day in KO dams. (No details were provided on when pup livers were weighed; the study report indicates that the liver of one pup/litter was weighed, and that the number of litters/group ranged from 6 to 14.) Absolute liver weight was increased at ≥1 mg/kg/day in WT dams and ≥3 mg/kg/day in KO dams (these data were not shown in the study report). Survival of WT pups from birth to PND22 was significantly reduced at 0.6 and 1 mg/kg/day; survival of KO pups was not affected. For HET litters,

survival was significantly decreased for HET pups born to WT dams (at 1 mg/kg/day) or KO dams (at 3 mg/kg/day).

Pup eye opening was delayed in a dose-dependent manner in WT pups, and at 1 mg/kg/day, the mean day of eye opening was significantly later than that of the control. At 1 mg/kg/day, WT pup body weights were significantly less than controls on PNDs 9, 10, and 22 for males and PNDs 7-10 and 22 for females. KO pup body weights were not affected. Post-weaning body weights of WT and KO mice were not significantly different from controls.

Under the conditions of this study, the reproductive/developmental toxicity NOAEL and LOAEL values appear to be 0.3 and 0.6 mg/kg/day, respectively, based on increased litter loss and decreased pup survival.[1] The maternal toxicity NOAEL and LOAEL values appear to be 0.6 and 1 mg/kg/day, respectively, based on increased liver weights in dams.

PFOA (Lau *et al.*, 2006)

Timed-pregnant CD-1 mice were dosed by oral gavage with 0, 1, 3, 5, 10, 20, or 40 mg/kg/day PFOA (>98% purity) in deionized water on GDs 1-17. Maternal body weight was monitored daily. Some dams were sacrificed on GD18 (45, 17, 17, 27, 26, 42, and 9 dams in the 0, 1, 3, 5, 10, 20, and 40 mg/kg/day exposure groups, respectively)[2], and the remaining pregnant dams received an additional treatment (same dose as on preceding days) on GD18 and were monitored hourly on GD19 for time of parturition (23, 8, 8, 19, 21, and 7 dams in the 0, 1, 3, 5, 10, and 20 mg/kg/day exposure groups, respectively)[3,4]. Blood and serum samples were collected from dams sacrificed prior to parturition, and livers were dissected and weighed; however, the study report did not provide liver-to-body weight ratios or liver histology data. The gravid uteri of these dams were excised and the numbers of resorptions and dead and live fetuses were recorded. Live fetuses were sexed, weighed, examined for external abnormalities, and then sacrificed and examined for either skeletal or visceral anomalies (1:1). Pups delivered by dams on GD19 were observed and the number of live pups, body weights, time of neonatal eye opening, and age of maturation (*e.g.*, vaginal opening in females and preputial separation in males) recorded. If fewer than 3 pups/litter were delivered, surviving pups were distributed to nursing dams within the same exposure group. Pup body weight and live pups/litter were tabulated daily for the first 4 days after birth and at intervals of "several" days thereafter.

There were statistically significant decreases in maternal mean body weight gain at 20 and 40 mg/kg/day and significant increases in absolute liver weight in dams of all treated groups (liver-to-body weight ratio data were not presented). There was no indication that livers were examined to determine if the increase in weight was accompanied by changes in liver histology.

Statistically significant increases in dams with litter resorptions were observed at ≥5 mg/kg/day, with 100% resorption at 40 mg/kg/day. Statistically significant decreases in the numbers of live fetuses

[2] Group sizes are based on the "Dams examined" field in Table 2 of Lau *et al.* (2006). The study authors did not indicate initial group sizes in the study methods, nor did they indicate whether mortality was observed in dams.
[3] The study authors did not indicate whether dams exposed to 40 mg/kg/day were partitioned to the group allowed to deliver.
[4] Group sizes are stated from the number of dams reaching parturition in Table 3 of Lau *et al.* (2006). The study authors did not indicate initial group sizes in the study methods, nor did they indicate whether mortality was observed in dams.

and fetal weights and a statistically significant increase in percentage of prenatal loss occurred at 20 mg/kg/day[5].

At 1, 3, and 20 mg/kg/day significantly increased incidences of enlarged fontanel were reported; percentages were 17.3, 66.7, 53.6, 18.2, 45, and 95 at 0, 1, 3, 5, 10, and 20 mg/kg/day, respectively. At 1, 3, and 20 mg/kg/day significantly increased incidences of reduced ossification of calvaria were reported; percentages were 13.5, 62.5, 66.7, 22.7, 35, and 55 at 0, 1, 3, 5, 10, and 20 mg/kg/day, respectively. The reduced ossification of calvaria and enlarged fontanel that occurred at the lowest dose was considered by this reviewer to be treatment-related, despite the absence of statistical significance at some intermediate doses and high variability in dose versus response. Additional significant effects on fetal development included reduced ossification of sternebrae, caudal vertebrae, metacarpals, metatarsals, and hyoid (20 mg/kg/day); reduced ossification of the proximal phalanges (1, 3,10, and 20 mg/kg/day); reduced ossification of supraoccipital and microcardia (10 and 20 mg/kg/day); and increases in tail defects (5, 10, and 20 mg/kg/day) and limb defects (5 and 20 mg/kg/day).

In delivered pups, significantly decreased neonatal survival was observed at ≥5 mg/kg/day, and significantly lower body weight was observed at ≥3 mg/kg/day. The study authors noted that this postnatal growth deficit corresponded to impairment of development as indicated by significant delay in eye opening (as much as 3 days) at ≥5 mg/kg/day. The text of the study implied that effects occurred on other developmental landmarks (*e.g.*, preputial separation) but data for these effects appeared to have been analyzed on a per pup basis.

The LOAEL for maternal toxicity was conservatively considered to be 1 mg/kg/day based on significantly increased absolute liver weights at all exposures levels (the study report did not provide liver-to-body weight ratios or liver histology data). The LOAEL for developmental toxicity was conservatively considered to be 1 mg/kg/day based on increases in the incidences of enlarged fontanel and reduced ossification of calvaria in pups; a NOAEL was not established. The increased number of dams with full litter resorption at ≥5 mg/kg/day may be considered either a developmental or a reproductive effect. If it is considered a reproductive effect, then the LOAEL and NOAEL values for reproductive toxicity would be 5 and 3 mg/kg/day, respectively.

APFO (Butenhoff *et al.*, 2004)

This study was reviewed in 2003 and included in the "U.S. EPA Preliminary Risk Assessment of the Developmental Toxicity Associated with Exposure to Perfluorooctanoic Acid and its Salts" (dated April 10, 2003)[6] and again in 2005 and included in the "U.S. EPA Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctanoic Acid and its Salts" (dated January 4, 2005)[7]. Relevant findings and conclusions of this study, as reported in the January 4, 2005 draft risk assessment, are summarized below. Please see the draft risk assessment for a full description of the study.

[5] The methods section of this study stated that statistical significance was determined by using the individual litter as the statistical basis.
[6] http://www nicnas.gov.au/Publications/NICNAS_Alerts/PFOs_Preliminary_Risk_Assessment_PNDF.pdf
[7] http://www.epa.gov/oppt/pfoa/pubs/pfoarisk.html

Groups of 30 Sprague-Dawley rats per sex, constituting the P generation, were exposed by gavage to 0, 1, 3, 10, or 30 mg/kg/day APFO (98% purity) in an EPA OPPTS 870.3800 guideline 2-generation reproductive study. P-generation rats were dosed daily at least 70 days prior to mating and until sacrifice. Two pups per sex per litter per group (60/sex/group) were selected for continued evaluation and dosing at weaning and constituted the F_1 generation. Of these F_1 rats, 30 mating pairs were randomly selected for mating, and non-selected pups were sacrificed and necropsied. Male rats were sacrificed after cohabitation and female rats were sacrificed on lactation day (LD) 22. Assessed reproductive endpoints included estrous cycling, sperm number and quality, mating, fertility, natural delivery, and litter viability and growth. Organs weighed in P and F_1 animals were brain, kidneys, spleen, ovaries, testes, thymus, liver, adrenal glands, pituitary, uterus with oviducts and cervix, left and right epididymis, prostate, and seminal vesicles. The pituitary, adrenal glands, vagina, uterus with oviducts, cervix and ovaries, right testis, seminal vesicles (with coagulating glands), right epididymis, and prostate were retained for histology. Histological examinations were performed on tissues from 10 rats/sex from the control and high-dose groups and for all gross lesions and reproductive organs from animals with reduced fertility in other dose groups. F_2-generation pup bodyweights were recorded on LD 1, 5, 8, 15, and 22, and anogenital distance was measured on LD1 and 22. Additionally, all F_2 males were examined for the presence of nipples. Further examinations in the F_2 were not indicated.

Reproductive endpoints were unaffected in both the P and F_1 animals receiving up to 30 mg/kg/day, the highest dose tested. Following exposure to 30 mg/kg/day, statistically significant developmental effects included decreased mean pup weight per litter in P-generation litters (F_1 pups) on post-natal days 1 through 15, and delayed sexual maturation (increased time to preputial separation and vaginal patency) in F_1 males and females. No statistically significant effects were reported in the F_2-generation. Systemic effects included lower mean body weight in P generation males exposed to doses of 3 mg/kg/day and greater and in F_1 males at all doses, increased absolute and relative (to body) liver and kidney weights in P and F_1 males at all doses, increased absolute and relative kidney weight (without microscopic changes) in P females at 30 mg/kg/day, and increased absolute pituitary weights (without microscopic changes; relative weight data not provided) in F_1 females exposed to doses of 3 mg/kg/day and greater. Hepatocellular hypertrophy and, less commonly, focal to multifocal hepatocellular necrosis were seen in the livers of F_1 males at 3 mg/kg/day and higher. No information was provided on whether or not histological changes were seen in the kidneys of P or F_1 males or in the livers of P males.

The LOAEL for systemic toxicity was 1 mg/kg/day (lowest dose tested) based on decreased mean body weight and increased absolute and relative liver weight in F_1 males, with evidence of liver histopathology at 3 mg/kg/day and higher in the F_1 males. The NOAEL for reproductive toxicity was the highest dose tested (30 mg/kg/day) and the LOAEL and NOAEL values for developmental toxicity were 30 and 10 mg/kg/day, respectively, based on decreased mean pup weight and delayed sexual maturation.

IV. SUPPLEMENTAL DATA

PFOA and APFO

Developmental toxicity studies by Gortner (1981), Gortner (1982), and Staples *et al.* (1984) were cited in two review articles on perfluoroalkyl acids by Lau *et al.* (2004 and 2007), and were also reviewed in 2003 and included in the "U.S. EPA Preliminary Risk Assessment of the Developmental Toxicity Associated with Exposure to Perfluorooctanoic Acid and its Salts"[6] and again in 2005 and

included in the "U.S. EPA Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctanoic Acid and its Salts"[7]. Brief summaries of these studies, based on information reported in the January 4 2005 draft risk assessment, are below. Please see the draft risk assessment for full descriptions of these studies.

Gortner (1981) administered time-mated Sprague-Dawley rats (22 per group) doses of 0, 0.05, 1.5, 5, and 150 mg/kg/day APFO in distilled water by gavage on GDs 6-15. Animals were sacrificed on GD20 and the ovaries, uteri, and contents were examined for the number of corpora lutea, number of viable and non-viable fetuses, number of resorption sites, and number of implantation sites. Fetuses were weighed and sexed and subjected to external gross necropsy. Approximately one-third of the fetuses were fixed and examined for visceral abnormalities. Remaining fetuses were subjected to skeletal examination.

Significantly reduced mean maternal body weights, ataxia, and three deaths were seen at 150 mg/kg/day. No significant differences between treated and control groups were noted for developmental parameters (including mean number of males and females, total and dead fetuses, the mean number of resorption sites, implantation sites, corpora lutea and mean fetus weights). The maternal toxicity NOAEL and LOAEL values were 5 and 150 mg/kg/day, respectively. The developmental toxicity NOAEL was 150 mg/kg/day, the highest dose level tested.

Gortner (1982) administered 0, 1.5, 5, and 50 mg/kg/day APFO in distilled water by gavage to 4 groups of 18 pregnant New Zealand White rabbits on GDs6-18. On GD29, does were euthanized and the ovaries, uterus and contents examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Fetuses were examined for gross abnormalities and placed in an incubator for a 24-hour survival check. Pups were subsequently euthanized and examined for visceral and skeletal abnormalities.

Significant transient reductions in body weight gain on GDs6-9 returned to control levels on GDs12-29. No clinical or other treatment-related signs were reported. No significant differences were noted between controls and treated groups for the number of males and females, dead or live fetuses, fetal weights, number of resorption and implantation sites, corpora lutea, the conception incidence, abortion rate, or the 24-hour mortality incidence of the fetuses. Gross necropsy and skeletal/visceral examinations were unremarkable. The only sign of developmental toxicity consisted of a dose-related increase in a skeletal variation, extra ribs or 13th rib, with statistical significance at 50 mg/kg/day (38% at 50 mg/kg/day, 30% at 5 mg/kg/day, 20% at 1.5 mg/kg/day, and 16 % at 0 mg/kg/day). The maternal toxicity NOAEL was 50 mg/kg/day, the highest dose tested. The developmental toxicity LOAEL was also 50 mg/kg/day.

Staples et al. (1984) conducted a developmental toxicity study of APFO which consisted of an inhalation and an oral portion, each with two trials or experiments. In the first trial the dams were sacrificed on GD21; in the second trial, the dams were allowed to litter and the pups were sacrificed on day 35 post-partum. For the inhalation portion of the study, the two trials consisted of 12 pregnant Sprague-Dawley rats/group exposed to 0, 0.1, 1, 10, or 25 mg/m^3 APFO for 6 hours/day, on GD6-15. In the oral portion of the study, 25 and 12 Sprague-Dawley rats for the first and second trials, respectively, were administered 0 and 100 mg/kg/day APFO in corn oil by gavage on GD6-15. Two additional groups (6 dams/group) were added to each trial that was pair-fed to the 10 and 25 mg/m^3

groups. On GD21, dams in trial one were sacrificed and examined for any gross abnormalities; liver weights were recorded and the reproductive status of each animal was evaluated. The ovaries, uterus and contents were examined for the number of corpora lutea, live and dead fetuses, resorptions, and implantation sites. Pups (live and dead) were counted, weighed, and sexed and examined for external, visceral, and skeletal alterations. The heads of all control and high-dose group fetuses were examined for visceral alterations as well as macro- and microscopic evaluation of the eyes. For trial two, in which the dams were allowed to litter, dams were sacrificed on day 23 post-partum (PP). Pups were counted, weighed, and examined for external alterations. Each pup was subsequently weighed and inspected for adverse clinical signs through day 22 PP. The eyes of the pups were also examined on days 15 and 17 PP for the inhalation portion and on days 27 and 31 PP for the gavage portion of the study. Pups were sacrificed on day 35 PP and examined for visceral and skeletal alterations.

In trial one of the inhalation study, treatment-related clinical signs of maternal toxicity occurred at 10 and 25 mg/m^3 and consisted of wet abdomens, chromodacryorrhea, chromorhinorrhea, and a general unkempt appearance. Three of 12 dams at 25 mg/m^3 died during treatment (on GD12, 13, and 17). Food consumption was significantly reduced at 10 and 25 mg/m^3; however, no significant differences were noted between treated and pair-fed groups. Significant reductions in body weight and increases in mean liver weights were observed at 25 mg/m^3. No effects were observed on the maintenance of pregnancy or the incidence of resorptions. Mean fetal body weights were significantly decreased at 25 mg/m^3 and in the control group pair-fed at 25 mg/m^3. However, interpretation of the decreased fetal body weight is difficult given the high incidence of mortality in the dams. Under EPA guidance, data at doses exceeding 10% mortality are generally discounted. In trial two of the inhalation study, clinical signs of maternal toxicity seen at 10 and 25 mg/m^3 were similar in type and incidence to those described for trial one. Two of 12 dams at 25 mg/m^3 died during treatment. Significant reductions in pup body weight were seen on day 1 PP. On days 4 and 22 PP, pup body weights continued to remain lower than controls, although the difference was not statistically significant. No significant effects were reported following external examination of the pups or with ophthalmoscopic examination of the eyes. Again, interpretation of these effects is problematic given the high incidence of maternal mortality.

For both trials of the inhalation study, the maternal toxicity NOAEL and LOAEL values were 1 and 10 mg/m^3, respectively, and developmental toxicity NOAEL and LOAEL values were 10 and 25 mg/m^3, respectively.

In trial one of the oral study, 3 of 25 dams at 100 mg/kg died during gestation (one death on GD11; two on GD12). Clinical signs of toxicity in dams that died were similar to those seen with inhalation exposure. Food consumption and body weights were reduced in treated animals compared to controls. No adverse signs of toxicity were noted for any of the reproductive parameters such as maintenance of pregnancy or incidence of resorptions. No significant differences between treated and control groups were noted for fetal weights or incidences of malformations and variations, nor were there any effects noted following microscopic examination of the eyes. In trial two of the oral study, similar observations for clinical signs were noted for the dams as in trial one. Likewise, no adverse effects on reproductive performance or in any of the fetal observations were noted. For both trials of the oral study, the maternal toxicity LOAEL and developmental toxicity NOAEL were both 100 mg/kg/day (the only dose level tested).

In an OPPTS 870.3700-compliant oral (drinking water) developmental toxicity study **(York 2000)**, pregnant Crl:CD rats (20/group) received target concentrations of 0, 0.01, 0.1, 1, or 30 mg/kg/day APFO in drinking water beginning 15 days prior to mating, and continuing through GD21 (the day of sacrifice). There were no deaths, clinical signs, significant treatment-related body weight changes, or treatment-related necropsy findings. There were no effects on number of corpora lutea, number of implantations, dead fetuses, resorptions, percent male fetuses, or fetal body weights. For fetal examinations, the only effect seen was a reduction in the average number of ossification sites per litter for sternebrae centers and for forelimb phalanges at 30 mg/kg/day; the study authors considered this to be a reversible developmental delay and not significant because it was not concentration-related and the incidences were within historical controls for the testing facility. Under the conditions of this study, the maternal and developmental toxicity NOAELs were 30 mg/kg/day, the highest concentration tested.

Lau *et al.* (2007) cited a study by **Wolf *et al.* (2007)** in which groups of pregnant CD-1 mice were administered 0 or 5 mg/kg/day APFO by gavage on GD7-17, 10-17, 13-17, or 15-17, or 20 mg/kg on GD15-17. Lau *et al.* (2007) reported that exposure to 20 mg/kg/day on GD15-17 produced neonatal mortality and birth weight reduction. At 5 mg/kg/day, birth weight reduction, growth deficits, and developmental delays were seen in mice exposed from GD7-17 or GD10-17 but not in those treated for shorter durations. In the same study, pregnant mice were treated with 5 mg/kg/day PFOA from GD1-17 and newborns were cross-fostered to control or treated dams. An increased incidence of neonatal mortality was seen in the *in utero* + lactational exposure group.

Lau *et al.* (2007) also cited a study by **White *et al.* (2007)** that investigated effects of PFOA exposure on mammary gland development in nursing mouse dams and their offspring. In this study, treatment with 5 mg/kg/day PFOA from GD1-17 did not affect the number of live pups born, but did impair postnatal growth similar to that described by Lau *et al.* (2006) and Wolf *et al.* (2007). A significant reduction in mammary differentiation among dams exposed to PFOA from GD1-17 or from GD8-17 was evident on PND 10, and delays in epithelial involution and alterations in milk protein gene expression were observed on PND 20.

In a neurobehavioral study of a single neonatal exposure of 10-day-old male mice **(Johansson *et al.*, 2008)**, PFOA exposure produced both increases and decreases in activity in the mice after 2 and 4 months. Ten-day-old male NMRI mice were administered a single dose of 0, 0.58, or 8.7 mg/kg PFOA by gavage in a 20% (w/w) fat emulsion. At the ages of 2 and 4 months, the mice were tested in a series of behavioral tests. At 2 and 4 months, administration of 8.7 mg/kg caused a decrease in locomotion, rearing, and total activity during the first 20 minutes of testing; at 2 months, administration of 0.58 mg/kg PFOA caused an increase in these parameters during the third 20-minute testing spell. At 4 months, administration of 0.58 mg/kg caused a decrease in locomotion and total activity; administration of 8.7 mg/kg produced an increase in locomotion, total activity, and rearing during the third 20-minute testing spell.

Fei *et al.* (2009)[8] examined whether exposure to PFOA may decrease fecundity in humans by measuring plasma levels of PFOA at weeks 4-14 of pregnancy among 1240 women recruited from 1996 to 2002. For this pregnancy, women reported time to pregnancy (TTP) in five categories (<1, 1-2, 3-5,

[8] Article provided to Bill Richards (SRC) by Andrea Pfahles-Hutchens (EPA) on 3/31/2009.

6-12, and >12 months). Infertility was defined as having a TTP of >12 months or receiving infertility treatment to establish this pregnancy. Longer TTP was associated with higher maternal levels of PFOA and this association was statistically significant.

Nolan *et al.* (in press)[8] examined associations between PFOA levels in cord blood and maternal plasma with lowered birth weight and gestational age in humans in a population of known high PFOA exposure. Residents drinking PFOA-contaminated water from the Little Hocking Water Association (LHWA) in Washington County, Ohio have serum PFOA levels approximately 80 times those in the general U.S. population. This study compared birth weights and gestational ages of neonates born to mothers residing in zip codes with water service provided completely, partially or not at all by the LHWA. The incidence of low birth weight, preterm birth, mean birth weight and mean gestational age of neonates did not significantly differ among water service categories. The authors concluded that markedly elevated PFOA exposure is not associated with increased risk of lowered birthweight or gestational age.

V. ASSESSMENT OF STUDY QUALITY

APFO (Abbott *et al.*, 2007)

The study report was missing some important details, namely group size. Additionally, Figure 1 in the study report dose not provide explanations for the lower-case letters above some bars in the figure. The study also does not identify a standard test guideline. Based on the number of adult females/group listed in the figure description for Figure 1, group number is highly variable (*e.g.*, 11-42 dams per dose group) and often below the 20 females/group with implantation sites at necropsy as recommended by OECD 414. The study authors did not provide a rationale for testing groups of different sizes.

PFOA (Lau *et al.*, 2007)

The study does not specify initial group size in the study methods, nor does it identify a standard test guideline. Based on number of dams examined in Table 2 and dams reaching parturition in Table 3, group number is highly variable (*e.g.*, 7-45 dams per dose group) and often below the 20 females/group with implantation sites at necropsy as recommended by OECD 414. The study authors did not provide a rationale for testing groups of different sizes.

APFO (Butenhoff *et al.*, 2004)

The study appeared to have been conducted according to OPPTS 870.3700 guidelines.

VI. REFERENCES

Abbott B.D., Wolf C.J., Schmid J.E., Das K.P., Zehr R.D., Helfant L., Nakayama S., Lindstrom A.B., Strynar M.J., & C. Lau. 2007. Perfluorooctanoic acid-inducted developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor alpha. Tox Sci 98(2): 571-581.

Butenhoff J.L., Gerald L.K., Frame S.R., O'Conner J.C., York R.G. 2004. The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology, 196: 95-116.

Fei C., McLaughlin J.K., Lipworth L., & J. Olsen. 2009. Maternal levels of perfluorinated chemicals and subfecundity. Human Reproduction 1(1): 1-6.

Case M.T., York R.G. & M.S. Christian. 2001. Rat and rabbit oral developmental toxicity studies with two perfluorinated compounds. Int J Toxicol 20: 101-109.

[redacted]. US EPA Administrative Record, AR-226 226-0463. As cited in the U.S. EPA "Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctanoic Acid and its Salts" (dated January 4 2005) available at http://www.epa.gov/oppt/pfoa/pubs/pfoarisk.html.

[redacted] US EPA Administrative Record, AR-226 226-0465. As cited in the U.S. EPA "Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctanoic Acid and its Salts" (dated January 4 2005) available at http://www.epa.gov/oppt/pfoa/pubs/pfoarisk.html.

Johansson N., Freedriksson A., & P. Eriksson. 2008. Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioral defects in adult mice. Neurotox 29: 160-169.

Lau C., Butenhoff J., & J.M. Rogers. 2004. The developmental toxicity of perfluoroalkyl acids and their derivatives. Toxicol and Appl Pharmacol 198: 231-241.

Lau C., Thibodeaux J.R., Hanson R.G., Narotsky M.G., Rogers J.M., Lindstrom A.B., & M.J. Strynar. 2006. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicological Sciences 90(2): 510-518.

Lau C., Anitole K., Hodes C., Lai D., Pfahles-Hutchens A., & J. Seed. 2007. Perfluoroalkyl Acids: A review of monitoring and toxicological findings. Toxicological Sciences, 99(2): 366-394.

Nolan L.A., Nolan J.M., Shofer F.S., Rodway N.V., & E.A. Emmett. In press. The relationship between birth weight, gestational age and perfluorooctanoic acid (PFOA)-contaminated public drinking water. Reproductive Toxicology.

Seed, J. 2000. Memo from J. Seed to Charlie Auer (EPA) dated August 31 2000 (subject: "Hazard Assessment of PFOS").

Staples R.E., Burgess B.A., and W.D. Kerns. 1984. The embryo-fetal toxicity and teratogenic potential of ammonium perfluorooctanoate (APFO) in the rat. Fundam. Appl. Toxicol. 4, 429–440. As cited in the U.S. EPA "Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctanoic Acid and its Salts" (dated January 4 2005) available at http://www.epa.gov/oppt/pfoa/pubs/pfoarisk.html.

U.S. EPA. 2003. Preliminary Risk Assessment of the Developmental Toxicity Associated with Exposure to Perfluorooctanoic Acid and its Salts. Dated April 10, 2003. Available at http://www.nicnas.gov.au/Publications/NICNAS_Alerts/PFOs_Preliminary_Risk_Assessment_PNDF.pdf

U.S. EPA. 2005. Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctanoic Acid and its Salts. Dated January 4 2005. Available at http://www.epa.gov/oppt/pfoa/pubs/pfoarisk.html

White S.S., Calafat, A.M., Kuklenyik Z., Villanueva L., Zehr R.D., Helfant L., Strynar M.J., Lindstrom A.B., Thibodeaux J.R., Wood C., et al. 2007. Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. Toxicol. Sci. 96: 133–144. As cited in Lau *et al.*, 2007.

Wolf C.J., Fenton S.E., Schmid J.E., Calafat A.M., Kuklenyik Z., Bryant X.A., Thibodeaux J.R., Das K.P., White S.S., Lau C.S., and B.D. Abbott. 2007. Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposure. Toxicol. Sci. 95: 462–473. As cited in Lau *et al.*, 2007.

[redacted]

Ecological Risk Assessment for P09-0291
Sara Pollack
May 12th, 2009

Draft Hazard Assessment: The results from Fish, Daphnid and Green Algae acute tests are considered valid. The hardness levels for both the acute fish and acute daphnid tests were far below the acceptable levels. This deficiency is not considered to affect the validity of the tests. All test concentrations were verified via HPLC analysis and found to be within > 20% agreement of nominal values therefore nominal values are reported. The concentration of concern (COC) is based on measured values for all three species showing effects at greater than 100 mg/L for each respective LC50 endpoint (>100 mg/L). To derive the COC, 100 mg/L is divided by a factor of 10 to simulate a chronic value, then divided again by an uncertainty (assessment) factor, yielding 1.0 mg/L or 1,000 ug/L (ppb). The PMN is of low concern for toxicity.

Exposure Assessment: The average surface water concentrations for PMN P09-0291 were determined by several probabilistic dilution model scenarios. The maximum concentration for water releases was [redacted] and the CC (1,000 ppb) was not exceeded for any of the projected estimated scenarios, imposing no acute or chronic risk to aquatic organisms.

Risk Characterization: There are no estimated risks associated with this PMN as it is due to the relatively low concentrations of potential water releases therefore the PMN substance P09-291 represents a low risk to aquatic organisms.

Testing Recommendations: Chronic testing is recommended for PMN P09-0291 if there is the possibility that the volumes released into the environment may increase and thus introduce greater surface water concentrations in the future. EPA recommends the following testing and stipulations:

Species	**EPA Test Guidance**	**Other parameters**
Fish Early Life-Stage	OPPTS 850.1400	Flow-through method, analytical measurement of test substance
Chronic Daphnia	OPPTS 850.1300	Flow-through method, analytical measurement of test substance
Algae	OPPTS 850.5400	Analytical measurement of test substance

In addition, we recommend submission to EPA of the proposed test protocols for review and comment (and modification if necessary) prior to initiating testing. EPA also recommends that a certificate of analysis be provided for the tested material.

POST FOCUS EXPOSURE REPORT (Standard Review)

Chemical ID: P090291 Reviewer: Kwon

Results Table: Dose, Concentration, and Days Exceeded Results Summary

Exposure Scenario[1]	Water						Landfill	Stack Air		Fugitive Air	
Release activity(ies)[2]; exposure calculation(s)[3]	Drinking Water		Fish Ingestion		7Q10[4] CC=1000 550	PDM Days Exceeded	LADD	ADR	LADD	ADR	LADD
	ADR	LADD	ADR	LADD							
	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	µg/l	# Days	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
[redacted] max ADR, max Acute Eco	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	---	[redacted]	[redacted]
[redacted] PDM1	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] PDM2	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] max LADD	---	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]: max ADR, max Acute Eco, PDM, max LADD	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] max ADR, max Acute Eco, PDM, max LADD	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	---	---

[1] Exposure scenario titles consist of release activity followed by exposure calculation abbreviation.
[2] Release activities are from engineering report's Manufacturing (Mfg), Processing (Proc) and Use release activity labels.
Multiple release activities are combined in one exposure scenario if their releases occur at same location.
[3] Exposure calculations are Acute Dose Rate (ADR), Lifetime Average Daily Dose (LADD), and Probabilistic Dilution Model (PDM). There may be one, two, or all three exposure calculations per exposure scenario.
CC is the aquatic concentration of concern.
[4] This column displays concentration values for the 7Q10 streamflow, which is defined as the average streamflow of the 7 consecutive days of lowest flow within a 10 year period.

Remarks:

Exposure Based Criteria/Persistent Bioaccumulative Criteria

Parameter	Exp Based	Persistent	Exceedance Value
Drinking (Surface) Water Dose (mg/kg/day)	No	No	
Fish Ingestion Dose (mg/kg/day)	No	No	
Inhalation Dose (mg/kg/day)	No	**Yes**	[redacted]
Groundwater Dose (mg/kg/day)	No	No	
Surface Water Release After Treatment (kg/yr)	No	No	
Total Release After Treatment (kg/yr)	No	No	

Fate test recommendations?: